National Institute of Diabetes and Digestive and Kidney Diseases
Workshop on Noninvasive measurement of Iron

Session II: Physical Properties of Iron

Chair:	Philip Aisen, Ph.D.	Albert Einstein College of Medicine
Panelists:	Alvin L. Crumbliss, Ph.D	Duke University
	Gary Buettner, Ph.D.	University of Iowa
	Denny N. Chasteen, Ph.D.	University of New Hampshire at Durham

Overview (Philip Aisen)

Iron in biological systems is found in a variety of oxidation states, reduction potentials, spin states, cluster sizes and reactivities. Each of these variables influences the detection and quantification of tissue iron.

About 3.5-4.0 grams of iron is present in the normal human subject. More than 70% of this iron is found in mononuclear form in the major oxygen-carrying heme proteins, hemoglobin and myoglobin. Some 20% of total body iron is present in stores, mostly in liver and almost entirely in polynuclear iron cores of ferritin . Heme- and nonheme-iron tissue enzymes account for perhaps 5% of total body iron, both mono- and polynuclear, while no more than 1-2% exists bound to circulating transferrin. The remainder is in the ill-defined but real and reactive "labile iron pool," generally thought to be iron in transit between stores, circulation and cellular enzymes.

The redox capabilities of iron underlie many of its biological activities, as well as its toxicity. Cellular uptake and excretion of iron, transmembrane transport and incorporation of iron into essential enzymes and into ferritin all depend on redox reactions. The one-electron reduction of dioxygen by Fe^{2+} results in superoxide formation, which in turn leads to the Fenton sequence generating hydroxyl radical, OHA, with its attendant toxicity.

In acid solution, pH <1.0, both ferrous and ferric iron exist as the hexaaquo complex, $[Fe(H_2O)_6]^{2+}$ or $[Fe(H_2O)_6]^{3+}$. When autoxidation is prevented by low oxygen tension, aquated ferrous iron persists throughout the pH range found in biological systems, but as the pH is raised above 2.0 the latter undergoes a stepwise series of hydrolytic deprotonations terminating in insoluble ferric hydroxide. Hydroxide complexes of iron readily polymerize by dehydration to form polynuclear complexes with iron atoms linked by oxo- or hydroxo-bridges. Iron polymers in biological systems may range from two iron atoms, as in the proteins with binuclear iron centers (e.g., ribonucleotide reductase and the purple acid phosphatases) to three-dimensional arrays of more than 4,000 iron atoms as in the mineralized core of ferritins.

Oxygen and Iron Toxicity (Garry Buettner)

Iron can be a detrimental catalyst in biological free radical oxidations. We hypothesize that the Fenton reaction with pre-existing H_2O_2 is only a minor initiator of free radical oxidations and that the major initiators of biological free radical oxidations are the oxidizing species formed by the reaction of Fe^{2+} with dioxygen. We employed EPR spin trapping to examine this hypothesis.

Free radical oxidation of: 1) chemical (ethanol, dimethyl sulfoxide); 2) biochemical (glucose, glyceraldehyde); and 3) cellular (L1210 murine leukemia cells) targets were examined when subjected to an aerobic Fenton ($Fe^{2+} + H_2O_2$) or an Fe^{2+}-dioxygen system. As anticipated, the Fenton reaction initiates radical formation in all the above targets. However, even without pre-existing H_2O_2, Fe^{2+} and O_2 also induce substantial target radical formation. When $[O_2]/[H_2O_2] < 10$, the Fenton reaction dominates target molecule radical formation; however, production of target molecule radicals via the Fenton reaction is minor when $[O_2]/[H_2O_2] > 100$. Interestingly, when L1210 cells are the oxidation target, $Fe^{2+} + O_2$ is observed to be responsible for formation of nearly all of the cell-derived radicals detected, no matter the $[O_2]/[H_2O_2]$ ratio. Our data demonstrate that when $[O_2]/[H_2O_2] > 100$, $Fe^{2+} + O_2$ chemistry is of major importance in the initiation of detrimental biological free radical oxidations.

Ferritin and its iron core (Dennis Chasteen)

The thermodynamic driving force for iron mineralization within ferritin is the production of highly insoluble iron(III)oxy/hydroxide phases. While the most dominant phase in vitro and in vivo is antiferromagnetic 6-line ferrihydrite, lesser amounts of the minerals goethite, hematite and maghemite are also observed in ferritin/hemosiderin from iron loaded tissues. Although maghemite may be present in small amounts, its ferrimagnetic properties can contribute disproportionately to the observed magnetism of tissues. Furthermore, it is unclear whether all of ferritin associated iron is contained within the protein cavity under conditions of iron overload since it is very difficult to load ferritin in-vitro beyond 2500 Fe/protein without some precipitation occurring. Protein surface bound iron and precipitated iron would influence the magnetism of the sample or tissue in a manner different from that of core iron.

The iron oxidation and hydrolysis reactions leading to the mineralization of ferritin and to the formation of reduced oxygen species have been determined in recent years. Hydrogen peroxide, oxyradicals and protein based radicals are produced during the oxidative deposition of iron in ferritin and probably play a role in the conversion of ferritin to hemosiderin and to the toxicity of iron. The ability of reductants to aid in mobilizing iron from ferritin/hemosiderin may further contribute to iron toxicity by making Fe(II) available for Fenton chemistry.

Redox and Coordination Chemistry of BioIron (Al Crumbliss)

In this contribution we will explore the fundamental coordination chemistry of Fe in the context of Fe mobility and storage in a biological system. Specifically, the interrelationship between the immediate chemical environment of Fe (inner coordination shell ligands) and the Fe(III)/Fe(II) redox potential will be described. The spin state, magnetic properties, degree of aggregation, solubility, mobility, redox potential, and kinetic and thermodynamic proclivity towards free radical generation are all directly dependent upon the immediate chemical environment of Fe ---that is, the donor atoms and ligands in the Fe inner coordination shell. We will examine the role of this dependence when Fe is transported and stored, and when it changes cellular compartments. An interdependence between first coordination shell

ligands and redox potential manifests itself in the ease of electron transfer and ease of ligand transfer, both kinetically and thermodynamically. Iron is likely to be found in the +3 oxidation state when tightly locked in place for purposes of transport or storage and the +2 oxidation state when a turnover of chemical environment is necessary.

These fundamental chemical concepts will provide the basis for viewing Fe(III/II) redox as a switch controlling the Fe storage/transport process and the coupling of ligand exchange to this redox as a synergism that amplifies the effect of the switch.

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